SYNOPSIS

Toxicity Induced changes of Cu and Pb on the Humoral and Cellular factors on *Anabas testudineus* (Bloch, 1792)

The unique physical and chemical properties of water enabled life to flourish and sustain human race for his development needs. As a consequence to this,increased industrialization, urbanization and population growth have contributed to progressive deterioration in the quality of earth's environment. Among all types of aquatic pollutants, heavy metals are of greatest concern due to their long half-life period, non-biodegradability, bioaccumulation and biomagnification properties. Aquatic pollution is a serious threat to the environmental quality that directly or indirectly affects the biological entities and ecosystem health. Intense industrial activities especially from the manufacturing and processing sectors during the last few decades have enhanced heavy metal pollution as well as contamination in water bodies and the biotic resources. The coastal ecosystems of Kerala, along with the unique backwaters are facing severe metallic and organic pollution problems affecting the very survival of biological communities in the region. The metal species of copper and lead have most serious environmental effects due to it's wide and rampant use in industrial,agricultural and other sectors of human activity. So understanding the toxic effects of these metals in the environment by developing suitable water quality standards is critical in suggesting specific management plans to revive and restore such affected coastal aquatic systems. In view of this,this Ph.D. thesis ***"Toxicity Induced changes of Cu and Pb on the Humoral and Cellular factors on Anabas testudineus (Bloch,1972)"*** is the following presented based on the following objectives.

1. To conduct acute toxicity tests of copper and lead on the fish, *Anabas testudineus* and assess the effective median lethal concentration.
2. To conduct chronic toxicity tests to assess the antioxidant activity in different chronic exposures of the metals.
3. To understand the changes in biomarkers - humoral,biochemical and histological in *Anabas testudineus* at sub-lethal concentrations of copper and lead due to metallic stress.
4. To determine the rate of accumulation of copper and lead in selected organs of the fish; the background concentration and also propose guidelines for managing such stressed environments.

The content of the thesis is distributed in six consecutive chapters. Chapter 1 deals with the **'General Introduction'** on heavy metal pollution, with special emphasis to haematological, biochemical and histological perspective. The relevant literature related to heavy metal pollution is elaborated.

Chapter 2 deals with **'Methodology in Toxicity Studies'**.Acute tests were derived from definitive tests at different concentrations for copper and lead,followed by static renewal bioassay. Subsequently LC_{50} values for 24 hours,48 hours,72 hours and 96 hours exposures for each metal was determined by Probit method and other standard protocols (APHA,2005,Finney,1971).The acute toxicity for copper (96 hour) was 1.74 mg/L and that for lead was 161.1mg/L.The chronic toxicity (sublethal) concentrations chosen for these metals were 0.34mg/L for copper and 32.33mg/L for lead ($1/5^{th}$ of 96 hour LC50 value),0.174mg/L for copper and 16.11mg/L for lead ($1/10^{th}$ of 96 hour LC50 value) and 0.113mg/L for copper and 10.74mg/L for lead ($1/15^{th}$ of 96 hour LC50 value). The chronic

exposures were also experimented with vitamin C to study the antioxidant properties for different durations of 7,14,21 and 28 days. Suitable controls were also maintained for the chronic tests. Appropriate tables and figures have been incorporated to support the data.

In chapter 3, **'Humoral Changes due to Copper and Lead'** is discussed.The detailed methodology for estimating different humoral parameters as well as the cellular changes of the fish subjected to different concentrations of chronic exposures are elaborated. Prominent response of copper exposed fish for 28 days showed occurrence of numerous amitotic,immature and hypochromic erythrocytes indicative of anaemia and hypoxia. In lead exposed fish,the blood parameters depicted a decrease which was parallel with increase in the duration of exposure. The leucocyte count significantly increased with increase in duration in both copper and lead treatments. Hepatotoxic biomarkers in blood,glutamate oxalate transaminase (GOT) and glutamate pyruvate transaminase (GPT) increased in both treatments.Serum lactate dehydrogenase (LDH) enzyme,cortisol and glucose level markedly increased in lead whereas in copper it decreased initially during the first 21 days and then increased up to 28^{th} day of exposure. Serum protein level decreased in the exposed fish in both the metal treatments. All the exposures were parallely done with vitamin C supplementation that obviously reduced the toxicity effects of the corresponding metal that was near to the control value justifying the prophylactic and curative role of the vitamin. All the chronic test results were statistically validated and found to be significant. Data evolved from the study were recorded and discussed with similar works in the area.Tables and figures have been incorporated.

The fourth chapter,**'Biochemical Changes due to Copper and Lead'** presents the variations in total protein,glycogen,LDH,GOT,GPT and antioxidant biomarkers,SOD and CAT in the liver, gill, kidney and muscle of heavy metal intoxicated as well as in vitamin supplemented fish. The protein content of liver decreased significantly from 68.69±0.58mg/gm to 37.59±0.17mg/gm and 73.37±0.43mg/gm to 51.29±0.03mg/gm and glycogen from 46.64±0.21mg/gm to 25.84±0.54mg/gm and 41.40±0.01mg/gm to 15.06±0.04mg/gm after 28 days in copper and lead intoxicated fish respectively. Similar decrease was observed in kidney,gill and muscle tissues. The magnitude of decrease in copper exposed fish was in the order gill > liver > kidney > muscle and in lead treated fish it was liver > gill > muscle > kidney. The percentage decrease in glycogen was maximum in liver followed by muscle in copper exposed fish whereas in lead, the percentage decrease was maximum in muscle followed by liver. Glycogen depletion was maximum in muscle than liver in copper exposed fish whereas in lead exposed the depletion was maximum in liver than muscle. Depletion of protein and glycogen in experimental fish justifies exhaustion of tissue metabolites as a consequence of oxidative stress. In liver, kidney and muscle,SOD increased significantly and consistently in all durations and doses of exposure of copper and lead. Catalase activity increased significantly in liver,kidney and muscle and a negative response was observed in the gill for lead and copper intoxicated fish. LDH, GOT and GPT registered consistent and significant increase in all exposures of copper and lead. Supplementation of vitamin C significantly decreased the activity of SOD and CAT in both copper and lead treated fish comparable to the control experiments. The test results were statistically tested and found to be significant. Tables and figures in support of the observation are adequately provided and the findings in the present study have been discussed in detail with similar works.

Chapter 5 deals with **'Histopathology and Bioaccumulation'**.Here liver,gill,kidney and muscle on chronic exposure to copper and lead noticed significant aberrations. Histopathological changes in copper and lead intoxicated fish were more or less

same. The prominent changes noticed in liver were necrosis, dilated congestion of sinusoids, hypertrophy and hyperplasia of bile duct, vascular degeneration etc. Copper and lead exposure produced rigorous histological changes in gill architecture. Besides general pathological changes such as lamellar swelling, epithelial rupture, secondary lamellar fusion and hyperplasia of branchial epithelium. Secondary changes were also observed comprising epithelial lifting, atrophy of gill lamellae, congestion in branchial blood capillaries, shortening and clubbing of secondary lamellae in higher sublethal concentrations and long exposures. Obvious histological changes noticed in the kidney and muscle in addition to liver and gill in heavy metal exposed fish justifies the efficiency of copper and lead in eliciting toxic response in fish. The microphotographs of histological sections unveiled the severity of damage of the organ studied. It was obvious from the study that,vitamin C treatment was effective in reducing the intensity of harm induced by both the metals. The results obtained in the present study was discussed in detail with similar works.

Trophic level accumulation of copper and lead in liver, gill, kidney and muscle of fish after 14 and 28 days of exposure at the corresponding sublethal concentrations of 0.34mg/L (Copper) and 32.22 mg/L (Lead) as well the bioaccumulation factor (BAF) were estimated. Copper depicted maximum accumulation in liver (34.063±0.230µg/gm) with BAF 100.394 followed by kidney,gill and muscle, that for lead exposed, the accumulation was highest in gill (860.005±0.002µg/gm) with BAF 26.719 followed by kidney,liver and muscle. In vitamin supplemented fish,metal accumulation reduced markedly compared to those exposed to copper and lead.The copper and lead concentration in water were higher during pre monsoon ie, summer period as compared to the post monsoon.

The 6th chapter, **'Summary and Conclusion'** discusses the outcome of the entire study so as to propose suitable water quality benchmarks (standards) specific to copper and lead in aquatic systems. The water quality monitoring programmes have great significance in pollution studies that draws parallel conclusions at the national level by the Central and State Pollution Boards,the Ministry of Environment and Forest and environmental protection agencies of United States and European regions. The use of antioxidant enzyme pathways in parallely deriving toxicant standards for the metals had been a hallmark of the investigation. The empirical and theoretical models developed based on the different toxicity tests derived through investigations could be a significant tool for the planners,policy makers and conservation specialists to regulate the fast increasing issues of metal contamination and general health. The relevant references for each of the chapters is incorporated in this section as **Bibliography.**